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NUTRITION REVIEWS

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NUTRITION REVIEWS

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MALNUTRITION AND EDEMA

The tendency for edema to develop among the populations of areas afflicted by famine commanded considerable attention in Central Europe during World War I. This famine edema was attributed by certain observers to lack of protein in the diet (A. V. Knack and J. Neumann, *Deutsch. med. Wchnschr.* **43,** *901* (*1917*); E. A. Kohman, *Am. J. Physiol.* **51,** *378* (*1920*); M. B. Maver, *J. Am. Med. Assn.* **74,** *934* (*1920*)).

Knack and Neumann (*loc. cit.*) by refractive indices detected low concentrations of protein in the plasma of the edematous subjects. Kohman (*loc. cit.*) in 1920 produced edema in rats by diets containing minimal amounts of protein. Besides confirming this, R. A. Frisch, L. B. Mendel, and J. P. Peters (*J. Biol. Chem.* **84,** *167* (*1929*)) showed that such rats developed serum protein deficiencies. These observations have been repeatedly confirmed, not only in the rat (H. Luckner, *Z. f. d. ges. exp. Med.* **103,** *563* (*1938*)), but also in the dog (R. Elman, F. A. Brown, and H. Wolf, *J. Exp. Med.* **75,** *461* (*1942*); S. A. Shelburne, *Arch. Path.* **17,** *152* (*1934*); A. A. Weech, E. Goettsch, and E. B. Reeves, *J. Exp. Med.* **61,** *299, 717* (*1935*); Weech and S. M. Ling, *J. Clin. Invest.* **10,** *869* (*1931*)). When the source of wastage can be checked and the normal protein content of the body restored, serum protein rises to its proper concentration (F. S. Bruckman, L. M. D'Esopo, and J. P. Peters, *Ibid.* **8,** *577* (*1930*)). In all these conditions serum albumin alone appears to suffer (Bruckman, D'Esopo, and Peters, *loc. cit.*; Shelburne, *loc. cit.*; Weech and Ling, *loc. cit.*)

The relation of hypoproteinemia to dietary protein deficiency *per se* has been challenged by A. L. Bloomfield (*J. Exp. Med.* **57,** *705* (*1933*); **59,** *687* (*1934*); **61,** *465* (*1935*)), who attributes it rather to the character of the diets employed, which consisted largely of carrots. That the degree of hypoproteinemia depends upon the nature as well as the quantity of protein in the diet has been demonstrated by others (Luckner, *loc. cit.*; J. Metcoff, C. B. Favour, and F. J. Stare, *J. Clin. Invest.* **24,** *82* (*1945*)). This accords with the fact that regeneration of serum albumin is promoted more by some kinds of protein than by others (S. H. Liu, H. I. Chu, S. H. Wang, and H. L. Chung, *Proc. Soc. Exp. Biol. Med.* **29,** *250* (*1931*); A. A. Weech, *Bull. Johns Hopkins Hosp.* **70,** *157* (*1942*)), vegetable proteins being in general less effective than animal proteins.

The relation of hypoalbuminemia to famine edema has also been questioned. Under standardized conditions in dogs, A. A. Weech, C. E. Snelling, and E. Goettsch (*J. Clin. Invest.* **12,** *193* (*1933*)) and Weech, Goettsch, and E. B. Reeves (*J. Exp. Med.* **61,** *717* (*1935*)) found a rather precise correlation. The concentrations of albumin in the serums of 24 edematous victims of famine in North China, who had subsisted for several months on diets consisting of corn flour, millet, and salted turnips, varied from 1.03 to 1.64 per cent (S. M. Ling, *Chinese J. Physiol.* **5,** *1* (*1931*)). The edema disappeared in every case when, in response to high-protein diets, serum albumin rose to the neighborhood of 2 per cent.

In 89 members of the civilian population of Saipan suffering from famine edema, studied by N. B. Kurnick (*Ann. Int. Med.* **28,** *782* (*1948*)), the concentration of total protein in the serum was less than 5.5 per cent in 69, above 6.5 per cent in only 5. J. B. Youmans (*J. Am. Med. Assn.* **99,** *883* (*1932*)) and Youmans, A. Bell, D. Donley, and H. Frank (*Arch. Int. Med.* **51,** *45* (*1933*)), on the other hand, in 31 edematous patients who had subsisted on diets containing in-

sufficient amounts of protein, found serum albumins between 1.72 and 4.97 per cent, averaging 3.85. Serum albumin was only moderately reduced also in a series of malnourished edematous infants studied by K. Dodd and A. S. Minot (*J. Pediatrics* **8,** *452 (1936)*).

A group of men who were given by A. Keys, H. L. Taylor, O. Mickelsen, and A. F. Henschel (*Science* **103,** *669 (1946)*) for three months diets containing 49 g. of protein per day, supplemented with vegetables, lost about 25 per cent of their body weight and developed obvious edema with only slight diminutions of serum albumin. L. Brull ("Les états de carence en Belgique pendant l'occupation Allemande, 1940–1944," *Editions Soledis, Liége (1945)*), P. Govaerts and P. E. Grégoire (*Acta Biol. Belg.* **4,** *530 (1941)*), and others reported only moderate hypoalbuminemia in famine victims in the Netherlands and Germany during World War II. Perhaps the most perplexing anomaly of all is the relatively normal concentrations of protein and of albumin found, in the face of extreme emaciation, in the serums of patients with anorexia nervosa (J. P. Peters and E. B. Man, *J. Clin. Invest.* **27,** *397 (1948)*) and in some with thyrotoxicosis (Peters, *unpublished studies*).

Despite these apparently discordant findings, it has been established by a large body of experimental work that serum albumin shares in the depletion of body protein produced by diets deficient in protein (A. A. Weech, M. Wollstein, and E. Goettsch, *J. Clin. Invest.* **16,** *719 (1937)*; L. A. Sachar, A. Horvitz, and R. Elman, *J. Exp. Med.* **75,** *453 (1942)*; W. J. Kerr, S. H. Hurwitz, and G. H. Whipple, *Am. J. Physiol.* **47,** *356 (1918)*). Furthermore, although malnutrition edema cannot be correlated with the degree of hypoalbuminemia, it is regularly relieved by administration of adequate amounts of protein and its discharge is accompanied by a rise of serum albumin. Starling's theory, based as it is on indisputable thermodynamic principles, stands as a yardstick by which explanations of edema must be evaluated. One reason for apparent discrepancies between concentrations of serum albumin and protein deficiency or edema is the procedures that have been commonly used for the fractionation of serum protein. The magnitude of the albumin deficit is minimized when proteins are fractionated by the conventional salting-out technics because, as albumin falls, alpha-globulin, which is in great part included with albumin in these procedures, increases (L. J. Zeldis, E. L. Alling, A. B. McCoord, and J. P. Kulka, *J. Exp. Med.* **82,** *157 (1945)*).

Both clinicians and physiologists should adopt procedures such as that of L. Pillemer and M. C. Hutchinson (*J. Biol. Chem.* **158,** *299 (1945)*) or the Milne-Majoor technic (J. Milne, *Ibid.* **169,** *595 (1947)*) that more precisely distinguish albumin from globulin. Measurements of the concentrations of total protein alone in the serum are obviously of little value. The actual mass of albumin in the circulation must be estimated not from the concentration of albumin in the serum, but from this concentration multiplied by the volume of circulating plasma.

H.-C. Chang (*Proc. Soc. Exp. Biol. Med.* **29,** *829 (1932)*) found that in malnutrition the blood volume, measured by means of carbon monoxide, varied with the concentration of proteins in the plasma. Kurnick (*loc. cit.*) has pointed out a similar reduction of plasma volume in the presence of edema in the subjects of Brull (*loc. cit.*) and of Keys *et al.* (*loc. cit.*). Contractions of circulating plasma volume in experimental animals as a result of dietary protein deficiency have been demonstrated by a number of observers (Metcoff, Favour, and Stare, *loc. cit.*; H. C. Torbert, *J. Exp. Med.* **62,** *1 (1935)*; Weech, Wollstein, and Goettsch, *loc. cit.*). They are to be expected since reduction of the oncotic pressure of the plasma favors transudation of fluid from the blood stream to the extravascular spaces. The colloid osmotic pressure of the plasma is only one of the determinants of the exchange of fluids between the blood

capillaries and the perivascular spaces. Under conditions conducive to retention of fluid, such as the administration of extra sodium chloride or sodium bicarbonate, edema will appear at unusually high concentrations of albumin (S. H. Liu, H. I. Chu, R. C. Li, and C. Fan, *Proc. Soc. Exp. Biol. Med.* **29,** *252* (*1931*); S. A. Shelburne and W. C. Egloff, *Arch. Int. Med.* **48,** *51* (*1931*); A. A. Weech, E. B. Reeves, and E. Goettsch, *J. Biol. Chem.* **113,** *167* (*1936*)).

The effect of hypoalbuminemia is enhanced by all factors that favor transudation, among which are certain disturbances of the circulation. Brull (*loc. cit.*) and others, in their studies of famine edema, have called attention to the presence of such circulatory disturbances. In states short of edema these same factors will promote hemoconcentration to mask albumin deficits. In the light of all these complicating factors, it should not be surprising that correlation between serum albumin, hypoalbuminemia, and edema has been established only under the most meticulously standardized conditions.

Attention has been called to another source of confusion. It has long been recognized that concentration of albumin in the serum of patients suffering from the effects of injury, operation, or infections is usually depressed. This depression has been rather widely attributed to wastage of body protein as a result of inadequate diets and destruction of tissues by injury or disease. Whereas serum albumin deficits of malnutrition appear only after loss of large amounts of protein, hypoalbuminemia is evident early in the course of pneumonia (J. K. Moen and H. A. Reimann, *J. Clin. Invest.* **12,** *589* (*1933*); R. A. Bruce and E. L. Alling, *Proc. Soc. Exp. Biol. Med.* **69,** *398* (*1948*)) and shortly after fractures (D. P. Cuthbertson and S. L. Tompsett, *Brit. J. Exp. Path.* **16,** *471* (*1935*)).

C. M. Grossman *et al.* (*J. Clin. Invest.* **24,** *523* (*1945*)) found that after operations and injuries and during acute infections, serum albumin often failed to diminish and sometimes even rose during periods in which patients lost large amounts of nitrogen in the urine. In these cases albumin was low at the beginning of the studies. In a subsequent series of experiments it was shown that serum albumin fell rapidly during the forty-eight hours immediately following operations, although excretion of nitrogen during the same periods was not large (J. P. Peters, *Ann. N. Y. Acad. Sci.* **47,** *327* (*1946*)). Similar reductions of protein had been noted by Cuthbertson and Tompsett (*loc. cit.*). The magnitude of these drops varied with the severity of the operation and the original concentration of albumin in the serum (Peters, *loc. cit.*).

The hypoalbuminemia of acute infections, operations, and injuries appears to be not a product of malnutrition, but part of the general disorder of metabolism that characterizes the reaction to injury and manifests itself also in "toxic destruction of protein." Like the latter, it is most evident in previously healthy and well-nourished individuals. If infection or injury become chronic and, through impaired appetite or digestion and increased catabolism, cause wastage of protein, the hypoalbuminemia of injury may give way to that of malnutrition.

Serum albumin deficits, as they are encountered in the clinic, can probably, with few exceptions, be attributed to either injury or malnutrition or both. The conditions in which they have been reported are innumerable (G. R. P. Aldred-Brown and J. M. H. Munro, *Quart. J. Med.* **4,** *269* (*1935*); H. G. Barbour and W. F. Hamilton, *Am. J. Physiol.* **73,** *315* (*1925*); J. S. Davis, *J. Lab. Clin. Med.* **21,** *478* (*1936*); V. P. Dole and K. Emerson, *J. Clin. Invest.* **24,** *644* (*1945*); L. R. Jones, *Am. Rev. Tuberc.* **23,** *325* (*1931*); Moen and Reimann, *loc. cit.*; J. P. Peters and A. J. Eisenman, *Am. J. Med. Sci.* **186,** *808* (*1933*); C. H. Webb, *Am. J. Dis. Child.* **44,** *1239* (*1932*); H. J. Wiener and R. E. Wiener, *Arch. Int. Med.* **46,** *236* (*1930*)). The nature, causes, and incidence of the injury reaction are still imperfectly defined. Attention has

been focused on practical, but not altogether fruitful, efforts to prevent the wastage of protein that it entails in order to accelerate convalescence and rehabilitation. It is possible that the injury reaction may have contributed to the hypoalbuminemia in those famine victims who had unusually large serum albumin deficits.

The incidence and nature of this reaction to injury require broader investigation. Uneconomic utilization of protein and hypoalbuminemia are not its only manifestations. The concentrations in the serum of alpha-amino acids and of lipids also fall sharply after injuries and operations (Peters, *loc. cit.*) and are usually reduced in acute infectious diseases (J. P. Peters and D. D. Van Slyke, "Quantitative Clinical Chemistry," *Vol. I, Ed. II, pp. 537 and 808, Williams & Wilkins Co., Baltimore (1946)*). They tend, like those of serum albumin, to remain depressed during the catabolic phase of protein metabolism.

The reductions of lipids, like those of albumin, have been generally attributed to malnutrition. Like albumin, the lipids appear to decline when nutrition becomes impaired (E. B. Man and E. F. Gildea, *J. Clin. Invest.* **15,** *203 (1936)*). Their variations, however, have received less attention than those of the serum proteins. Undoubtedly, these are not the only chemical derangements in malnutrition and after injury. It has been discovered that serum-precipitable iodine, though in general little influenced by nutritive disorders *per se*, falls to concentrations characteristic of myxedema when serum albumin is extremely diminished (Peters and Man, *loc. cit.*). These examples merely illustrate a descriptive approach that may aid in the definition and differentiation of the effects of malnutrition and injury and thereby to the elucidation of the nature and cause—and possibly the rectification—of the reaction to injury.

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VITAMIN B_6 DEFICIENCY IN THE HAMSTER

Descriptions of the various sequelae of vitamin B_6 deficiency in the rat, dog, chick, pig, monkey, and mouse have been recorded by several investigators. No detailed description of the results of this deficiency in the Syrian hamster has, however, previously appeared.

G. Shwartzman and L. Strauss (*J. Nutrition* **38,** *131 (1949)*) have described such experiments. Weanling hamsters were placed on a vitamin B_6-free ration adequate in protein, salts, and other vitamins, and containing either corn oil or hydrogenated cottonseed oil. Control groups received 50 micrograms of pyridoxine daily by subcutaneous injection. All animals gained in weight for about two weeks. With longer time, the control group continued to gain weight and appeared active and healthy, whereas the deficient group either maintained or slowly lost weight. Little difference was apparent between those deficient animals which received corn oil and those which received hydrogenated cottonseed oil, although the former group maintained slightly higher weights than did the latter, and were considerably slower in showing the onset of additional symptoms of vitamin B_6 deficiency.

These additional symptoms included a greatly enhanced excretion of xanthurenic acid (from 10 to 15 mg. per 100 g. of ingested protein in the control group to a high of 306 mg. in the deficient group during the eighth week), a change in the normal velvety texture of the fur to a lustreless gray-brown color with some thinning of the fur and alopecia, a change from the normally active

state to one of sluggishness, with a loss of fat deposits. The deficient animals survived for weeks in this condition, but eventually died after twelve to thirteen weeks on the deficient ration. Autopsy showed loss of fat tissue and marked atrophy of the thymus and other lymphoid tissue even when the external signs of vitamin B_6 deficiency were mild. There was an arrest of sexual maturation and bone growth.

The plateauing in growth of the deficient animals began simultaneously with a markedly decreased food intake, and to determine to what extent the lowered food intake was responsible for the deficiency symptoms, a control group was given pyridoxine subcutaneously, but was regulated in its food intake so as to keep the weight at a level with that of the deficient animals. Such animals maintained their normal fur, muscle tone, and activity, in contrast to the deficient group, and merely appeared younger than their mates fed ad libitum. Although atrophy of lymphoid tissue did occur in these controls, it was less severe than in the deficient group of animals.

All of the symptoms of vitamin B_6 deficiency evident after a depletion period of nine and one-half weeks were alleviated by injection of pyridoxine hydrochloride. The appetite returned to normal, the fur changes were rapidly repaired, and growth resumed. Fat deposits reappeared in the liver. None of these changes was observed following addition of extra corn oil to the deficient ration.

Except for differences in detail, which may result as much from dietary differences as from specific differences, the symptoms of vitamin B_6 deficiency observed in the hamster thus resemble, in general, those observed in many other animal species. No studies of the quantitative requirement for vitamin B_6, of its dependence on the nature of the ration, or of the comparative activities of the different forms of this vitamin were made. The study indicates that in this, as in other animal species, vitamin B_6 is somehow involved in fat metabolism. What its role here may be, and whether it is a primary role in synthesis and metabolism of fatty substances, or whether it is secondary to the now well-established role of vitamin B_6 in protein metabolism is still a mystery.

EFFECT OF DIET DURING TOOTH DEVELOPMENT

Clinical and experimental studies which demonstrated strong relations between the composition of the diet during tooth development and the susceptibility to tooth decay have been reviewed previously (*Nutrition Reviews* **6,** *196 (1948)*). Since that time, R. F. Sognnaes (*Am. J. Dis. Child.* **75,** *792 (1948)*; *J. Am. Dent. Assn.* **37,** *676 (1948)*) has presented more detailed reports of the above relations which strengthen the earlier conclusions.

The clinical survey of trends of dental caries incidence which have occurred in European children during the past fifty years was based on the examination of about 800,000 children by various investigators in eleven European countries: Czechoslovakia, Denmark, England, Finland, France, Germany, Holland, Norway, Scotland, Spain, and Sweden. Varying degrees of reduction in the dental caries incidence were observed during both World Wars I and II for groups of children from these countries. However, these reductions were not immediate and did not reach a maximum until after five or six years of war regimens. Sugar rationing began in these countries before or almost immediately after the beginning of hostilities and then remained constant or, in some cases, even increased slightly toward the end of the war. With the reduction of sugar, there was at the same

time a great decrease in other refined carbohydrates and proportionate increases in the use of flour of high extraction and vegetables.

The wide interval of time between the decrease in refined carbohydrates and the reduction in dental caries incidence could not be satisfactorily explained on the basis of a rapid or concurrent mechanism operating through the oral environment. Actually, the length of time necessary to produce the maximum reduction in tooth decay corresponded reasonably well with the length of time needed for the complete development of the teeth. These facts strongly suggested that the wartime reduction in tooth decay could best be explained by an indirect favorable influence on the development and maturation of the teeth and could not be attributed solely to a change in the oral environment.

In the studies with mice, hamsters, and the Long-Evans strain of rats, carious lesions did not develop to any appreciable degree in animals which were fed a diet composed of natural foodstuffs. When the offspring of females fed the natural diet throughout pregnancy and lactation were maintained after weaning on caries-producing purified rations, they developed only a few carious lesions.

Tooth development is largely complete in the above species when the offspring are weaned at the normal age of 21 days. However, when females of these species were fed the natural ration throughout pregnancy but were transferred to the purified ration at the time their litters were born, and the offspring fed purified rations after weaning, these offspring had a higher dental caries experience than those in the previous group. When dams of these species were fed the purified ration throughout pregnancy and lactation and the offspring continued on the same diet after weaning, these rodents had an even higher susceptibility to tooth decay. For example, the ratio of dental caries incidence, as expressed in terms of caries score per month of posteruptive oral exposure, of three groups of hamsters whose exposure to the caries-producing ration had begun during prenatal life, at birth, or at weaning (that is, before, during, and after tooth development) was 20:10:2.

G. Toverud (*Brit. Dent. J.* **86**, *191* (*1949*)) has reported the results of a series of surveys to determine the effect of general health supervision on the frequency of dental caries in groups of Norwegian children. Two communities were included in these studies: the first a rural community, Skedsmo, where the survey began in 1936; and the second, one fourth of the city of Oslo where observations were begun in 1939. Health supervision began in the prenatal period and was also undertaken with children who were as old as 7 years. An important phase of the program was instruction of the mothers in the right choice of foodstuffs and in methods for suitable preparation of food for themselves and their offspring. The diets recommended were such as would supply nutritional requirements during the various periods of growth and reproduction. A special effort was made with respect to calcium, iron, protein, and vitamins A, D, and the B-complex. Where it was deemed necessary, extra vitamin C was recommended. Vitamin K was given to all pregnant women during the last three weeks before the expected date of delivery. Thiamine excretion was checked in pregnant women and synthetic thiamine was given where indicated. Whole wheat cereals were offered to the children beginning in the sixth month of life. Vegetables were emphasized in both raw and cooked forms. The recommended diets in every case were low in sugar, sweets, cookies, and pastries.

'The over-all health-supervision program for pregnant women appeared to be beneficial, as demonstrated by comparison of the stillbirth rate and the infant mortality rate observed among outpatients at the health station in Oslo with the same rates in the city of Oslo as a whole. For the years

1939 to 1946 in the entire city of Oslo, 29,191 babies were born, of which 688 (23.2 per 1000 births) were stillborn; of the 28,503 live infants, 768 (26.9 per 1000 live births) died during the first year. In contrast, the infants of women instructed at the health station in Oslo totaled 1553 in the years 1939 to 1947 of which 22 (14.2 per 1000) were stillborn; of the 1531 live births 18 (11.8 per 1000) died during the first year of life. These data appear to be the more significant because such a difference could be demonstrated despite wartime regimentation and restriction.

The dental examinations of the children were grouped by years of age into five categories: $2\frac{1}{2}$ to 3, 3 to 4, 4 to 5, 5 to 6, and 6 to 7 years. The dental caries incidences of the individual age groups were compared from year to year. In other words, the investigator compared the dental caries experience of a given age group of children with that of children of the same age in any preceding or subsequent year for which data were available. In the community of Skedsmo, 78.3 per cent of 23 children, $2\frac{1}{2}$ to 3 years of age, in the years 1936 and 1937 had tooth decay; 27.1 per cent of their teeth were carious. However, in 1940 only 44.2 per cent of 43 children of the same age had dental caries and only 13.1 per cent of their teeth were carious. In children of this age group, the elapsed period of four years of health education was sufficient to cover the entire period of tooth development, i.e., throughout pregnancy and postnatal life. Of 88 children, 3 to 4 years of age, in the years 1936 and 1937, 92.0 per cent had some degree of tooth decay; 45.5 per cent of their teeth were carious. In 1940, 83.8 per cent of 37 children had tooth decay and 36.1 per cent of their teeth were diseased. In the three still older groups of children, there were no significant decreases in the dental caries experiences. Obviously, the older children did not come under the general health-supervision program until after the teeth were largely or completely developed. Any alteration in the diet after this time would be effective in producing variations in the maturation, maintenance, and oral environment of the teeth and not as an influence during the major phases of development and calcification.

For the children in Oslo, the data were presented in somewhat different style; instead of comparison of age groups on a yearly basis, the data were only classified in accordance with whether the children registered with the health station before 1 year of age or after 1 year of age. In each of the four age groups between $2\frac{1}{2}$ and 6 years, there was significantly less tooth decay among the children admitted for health supervision at the earlier age. For the 6 to 7-year-olds, there was a slight but statistically insignificant lower degree of tooth decay among these children admitted at less than 1 year of age. Unfortunately, no data were available for children in the same district in Oslo prior to the beginning of the health station, nor for those in the same period who were not under health supervision.

Just as this survey was reaching a stage where valuable data were becoming available, the war and German occupation drastically altered the Norwegian dietary and thus broke the continuity of the investigation. The full significance of these studies, in so far as permanent teeth are concerned, needed a much more prolonged continuation of the observation period. These projects had been under way barely long enough to give a picture of what health supervision might do to reduce the susceptibility to tooth decay in the deciduous teeth, and not nearly long enough to give any suggestion of the effect upon the permanent teeth when war began with drastically altered dietary patterns.

In Skedsmo, the health-supervision project was continued throughout the war but dental examinations were discontinued from 1941 to 1943, inclusive. In 1944, when the dental survey was begun again, the data

showed that the children were remarkably less susceptible to tooth decay than children of the same age had been in 1940. This decrease was comparable to those reported from other communities and countries for wartime periods. A still further decrease in dental caries experience was visible in 1945 for all ages and again in 1946 for the 5 to 6 and 6 to 7-year-old groups. In view of general wartime caries reductions, it is impossible to determine how much, if any, of the decreases after 1940 in Skedsmo children were attributable to the health-supervision program.

Toverud suggested that the reduction in tooth decay could have been due to all or any combination of three possibilities: "(1) A supervision of the general health of the mother and child. (2) Regulation of the diet for the mother and child so as to meet the requirements for growth and maintenance. (3) Regulation of the diet and habit of living so as to reduce the local caries factors as much as possible." In the discussion of the pros and cons for each of the three premises, he emphasizes his viewpoint on the value of excluding sugar and sweets from the diet in the following statements: "A more natural explanation of the cause of the decreased difference is given through the third factor" and "Besides acting as a local factor, uncontrolled indulgence of sugar and sugar products may also alter—reduce—a good resistance laid down during the pre-eruptive period."

A preliminary series of investigations on the relation of diet during tooth development to the susceptibility to dental caries has been conducted with hamsters of various ages by D. F. Mitchell and W. G. Shafer (*J. Dent. Res.* **28,** *424 (1949)*). The standard breeding ration was the same natural foodstuff diet used in the investigations of Sognnaes (*loc. cit.*). However, in this case, the caries-producing diets were of rather crude composition instead of the highly purified type used by the previous investigator. The first diet was composed of: whole wheat flour 30 per cent, corn starch 20 per cent, confectioner's sugar 15 per cent, whole powdered milk 30 per cent, alfalfa 4 per cent, and sodium chloride 1 per cent. The second diet was similar to the first except that the level of confectioner's sugar was reduced to 10 per cent, the amount of alfalfa to 3 per cent, and the whole wheat flour dispensed with; all reductions were compensated for by an increase in the corn starch from 20 to 56 per cent.

In the first experiment, the first diet was offered to the hamsters at various stages in development. Eleven pregnant female hamsters were used which bore 53 offspring. Seven of the female hamsters were transferred to the caries-producing diet at varying periods from one to twelve days before parturition. Three of the 4 remaining females and their litters were transferred to the caries-producing diet when the offspring were 4, 8, and 14 days of age. Litters of these 10 females were still maintained on the caries-producing diet after weaning. The litter of the eleventh hamster was not transferred from the natural breeding ration to the caries-producing diet until 30 days of age. The experimental exposure to the caries-producing diet was ninety days for each litter including the prenatal period. In this way, although the total experimental period was identical for all groups, the length of time that the teeth were exposed through the oral environment to the caries-producing diet varied between groups. For example, the actual exposure to the oral environment was shortest for those animals where the diet was begun prenatally but was the full ninety days for the animals where the diet was begun at 30 days of age when tooth development is largely complete. The highest dental caries experience among those hamsters was observed among the animals which were transferred to the caries-producing diet at 8, 14, and 30 days of age; the next highest were the offspring of the hamster transferred to the diet at twelve days before parturition

even though the oral exposure was the shortest of all groups. The rest of the animals had a uniformly low degree of tooth decay.

In the second experiment, the second caries-producing diet was administered to 2 female hamsters at fifteen days before parturition and to the offspring of 2 other hamsters beginning 20 and 22 days after birth, respectively. Again the total experimental period was ninety days. In this case the offspring of the hamsters which were maintained on the diet during pregnancy had a much higher incidence of tooth decay than the hamsters transferred to the diet at weaning.

An interesting point which was not commented upon by the investigators was that the average incidence of tooth decay among the hamsters fed the second caries-producing diet for ninety days after weaning at 20 and 22 days was less than 10 per cent of that observed in hamsters on the first caries-producing diet for ninety days after beginning at either 14 or 30 days of age. This difference certainly raises two questions which need definite and conclusive answers based on sufficient experimental data: How do the caries-producing properties of these two diets compare in animals whose teeth are complete at the beginning of the experimental period, and how predictably and uniformly susceptible to tooth decay were the experimental animals which were used herein when the prenatal and early postnatal periods were kept identical? On the basis of the limited data and even seemingly conflicting results mentioned above, there does not seem to be justification for a conclusion that the diets during tooth development had increased the susceptibility to tooth decay.

Both the clinical and laboratory investigations described above demonstrate the difficulties inherent in the study of the effect of diet during tooth development on the caries susceptibility of the teeth at maturity. The suggestion that the developmental diet can contribute to resistance or susceptibility to tooth decay is sufficiently strong to merit intensive investigation for the actual factors and mechanisms which mediate these effects.

ARTERIOSCLEROSIS, CHOLESTEROL, AND OBESITY

Arteriosclerosis is not only a medical problem of prime importance but also one with many nutritional facets. In a previous review on the subject (*Nutrition Reviews* **7,** *155* (*1949*)) it was indicated that the basic pathologic lesion in arteriosclerosis was infiltration of the intima and subintima of the arterial wall with lipoids, consisting, in part, of cholesterol. Calcium may then be laid down and in time the continuity of the intimal wall may be broken so that thrombosis occurs. The lipoids may enter the vessel wall directly from the vessel, passing into the intima and subintima, or they may arrive via the blood supply of the vessel wall. The reasons for the deposition of lipoids and cholesterol in the walls of blood vessels are not known.

Most of the experimental work with arteriosclerosis has been in animals but there is considerable doubt whether the production of arteriosclerotic lesions in small animals that never spontaneously develop the disease can be compared with the human condition.

M. Faber (*Arch. Path.* **48,** *342* (*1949*)) has contributed some pathologic and biochemical studies with respect to arteriosclerosis in the human aorta. Fifty human aortas were studied by analysis of the total sulfate and cholesterol content. The cholesterol content was taken as a measure of the lipoid contained in the aorta and the sulfate was thought to correspond with the quantity of polymerized carbohydrate-sulfuric esters, such as are found in various

parts of the body as heparin, chondroitinsulfuric acid, hyaluronic acid sulfate, etc. In the aortas from normal individuals there was a gradual increase with age in sulfate and in cholesterol content. There seemed also to be an increase with age in these two substances in the aortas of individuals with high blood pressure and with syphilitic infection of the aorta, but the striking thing with respect to the aortas in these two diseases was that both the sulfate and cholesterol contents were in most instances far above those found at any age in the normal individuals. Two patients with xanthomatosis and increased amounts of cholesterol in their aortas did not show an increase in sulfate content.

In discussing the relation between the carbohydrate-sulfuric esters (as measured by sulfate content) and arteriosclerosis, the author points out that although cholesterol is present in all cells it forms extracellular deposits only in certain parts of the body, such as cartilage, the normal aorta, the hypertensive aorta, and in acute inflammatory tissues. Such extracellular deposits occur only where carbohydrate-sulfuric esters are likewise found. Some of the esters in these tissues have actually been isolated; in other instances their presence in the tissues has been assumed because of characteristic staining reactions.

Although many clinical, experimental, and statistical factors have pointed to a possible relation between obesity and arteriosclerosis for many years in the past, no conclusive data have been drawn up either to prove or disprove such a relation. This matter has been discussed (*Nutrition Reviews* **6,** *99 (1948)*) and it was pointed out that a study by S. L. Wilens in New York indicated a relation of overnutrition as measured by body weight and the frequency and severity of aortic sclerosis. Further work in this regard has been reported by M. Faber and F. Lund (*Arch. Path.* **48,** *351 (1949)*). These authors were critical of the studies made before in that the degree of aortic sclerosis was measured only by inspection, and thus only roughly graded as to the relative severity. In the series of aortas studied by Faber and Lund the degree of arteriosclerosis was measured by the sulfate and cholesterol contents of the aorta. No relation between these values and obesity could be found. Thus, although there was a clearcut relation between obesity and hypertension and thus between obesity and the arteriosclerosis complicating the hypertension, when the patients with hypertension were eliminated, obesity was no longer related to the degree of arteriosclerosis in the aorta.

Unquestionably, much more work must be done before even these simple matters with relation to arteriosclerosis in man can be settled. Nevertheless, here are some accurate biochemical measurements relating arteriosclerosis, chondroitinsulfuric acid, and hyaluronic acid with hypertension and chronic inflammation but not with xanthomatosis. It is unfortunate that only a few serum cholesterol concentrations were determined in this study for it is upon studies of blood concentrations that the clinical investigator must depend as a rule for observation of a therapeutic effect.

USE OF METABOLIC INHIBITORS IN STUDIES WITH DEVELOPING CHICK EMBRYOS

Antivitamins related in structure to the true vitamins have proved useful tools in the study of the course of metabolic reactions in microorganisms, and in higher animals. Examples of such studies have been provided in numerous reviews (*Nutrition Reviews* **6,** *153, 313, 338 (1948)*).

Several articles dealing with application of

these substances to the developing chick embryo have appeared. In the first of these (W. W. Ackermann and A. Taylor, *Proc. Soc. Exp. Biol. Med.* **67,** *449 (1948)*), the effects of 3-acetylpyridine were investigated. This compound, an analogue of niacin, was previously reported to produce pellagra-like symptoms when administered to mice. These effects were nullified by feeding either niacin or its precursor, tryptophan (D. W. Woolley, *J. Biol. Chem.* **162,** *179 (1946)*), together with the analogue.

In the present investigation, varied amounts of 3-acetylpyridine with or without nicotinamide, niacin, or tryptophan were injected into 4-day-old eggs. Injections of graded doses of the inhibitor established that 600 micrograms produced 100 per cent mortality of the embryos within twenty-four hours. However, even 6000 micrograms of the inhibitor were nontoxic when injected together with sufficient nicotinamide. Experiments at several different concentrations established that the relation between acetylpyridine and nicotinamide was fully competitive, the ratio of the inhibitor (acetylpyridine) to metabolite (nicotinamide) at which toxic effects of the inhibitor were just nullified being approximately 14 to 1. In comparison, niacin and tryptophan were much less effective. Twenty-five hundred micrograms of niacin failed to nullify completely the toxic effects of 2000 micrograms of the inhibitor, whereas only 100 micrograms of nicotinamide were required for this purpose. Seventy-five hundred micrograms of tryptophan were required to nullify the toxic effect of only 700 micrograms of acetylpyridine. Since both tryptophan and niacin fully counteracted the effects of acetylpyridine only at low levels of the latter, whereas nicotinamide was effective at much higher levels, it was concluded that the ability of the 4-day-old chick embryo to transform the acid to the amide is limited.

It is thus apparent that the toxic effects of acetylpyridine to the chick embryo result from production of a lethal nicotinamide deficiency, which can be counteracted by injection of nicotinamide. If sublethal doses of the inhibitor are added, or if lethal doses are rendered barely sublethal by injection of nicotinamide, a maldevelopment of the chick occurs. This was characterized by undersized and deformed legs, and by a generally edematous condition at the body surface.

Somewhat similar studies employing desoxypyridoxine, an inhibitor which counteracts the action of vitamin B_6 in chicks, rats, and dogs, have been reported by W. W. Cravens and E. E. Snell (*Proc. Soc. Exp. Biol. Med.* **71,** *73 (1949)*). Injection of 500 to 1000 micrograms per egg of this compound produced 100 per cent mortality of the embryos. Almost all of these deaths had occurred before the sixth day of incubation. That this mortality was produced by interference with the essential role of vitamin B_6 was demonstrated by the observation that simultaneous injection of 1000 micrograms of pyridoxal completely prevented the toxicity. Pyridoxamine and pyridoxine also were effective in counteracting the toxicity of the inhibitor. The ratio of inhibitor (desoxypyridoxine) to vitamin B_6 which permitted 50 per cent of the embryos to survive was found to be about 1 to 20 for pyridoxal, 1 to 50 for pyridoxamine, and 1 to 100 for pyridoxine. Other compounds injected included niacin, nicotinamide, and DL-alanine. None of these compounds had any effect on the toxicity of desoxypyridoxine.

As incubation progressed, the chick embryo became more resistant to intoxication with desoxypyridoxine. After four or more days of incubation, 1000 micrograms of the inhibitor did not produce any embryonic deaths. Higher levels of the inhibitor were toxic, but this toxicity was apparently not related to its effects as an antivitamin, since it could not be counteracted by any of the three forms of vitamin B_6. Indeed, pyridoxal and pyridoxine were themselves toxic at similar levels (5 to 10 mg. per egg). Results of the study again show how metabolic in-

hibitors can be applied to a study of chemical embryology. It had not been possible previously to ascertain the role of vitamin B_6 in embryonic development, since hens fed diets deficient in vitamin B_6 stop laying before sufficient eggs are obtained to carry out the necessary experiments. Results with the inhibitor show unmistakably that the need for vitamin B_6 becomes critical early in embryonic growth.

Finally, D. A. Karnofsky, P. A. Patterson, and L. P. Ridgway (*Proc. Soc. Exp. Biol. Med.* **71,** *447 (1949)*) have tested the effects of folic acid and many related compounds, including several antagonists of folic acid, on development of the chick embryo. Injections were made after four days of incubation. Amounts of folic acid (pteroylglutamic acid), pteroyldiglutamic acid, and pteroyltriglutamic acid up to 20 mg. per egg were essentially nontoxic. By contrast, the LD_{50} for 4-aminofolic acid (aminopterin), for 4-amino-N^{10}-methylpteroylglutamic acid, 4-amino-9-methylpteroylglutamic acid, and 4-amino-9,10-dimethylpteroylglutamic acid ranged between 3 and 5 micrograms per egg. Several other folic acid antagonists were less toxic.

The effects of these antagonists on the embryos were striking: severe stunting occurred in all cases; other abnormalities included a flattened head, small eyes, absent or underdeveloped lower beak, etc., the exact changes depending upon the age of the embryo at death. From the structural similarity of these inhibitors to folic acid, it is tempting to ascribe these results as due to interference with folic acid function by the toxic compounds. However, such an interpretation, though it appears likely, must still be made with caution, since the toxic effects of the analogues could not be prevented by simultaneous injection even of large amounts of folic acid. Difficulties in counteracting the toxic effects of aminopterin in other species with folic acid also have been observed, although the evidence indicates that the effects of this compound are predominantly due to its interference with folic acid metabolism (A. L. Franklin, E. L. R. Stokstad, and T. H. Jukes, *Proc. Soc. Exp. Biol. Med.* **67,** *398 (1948)*).

The developing chick embryo is a favorable subject for biochemical and nutritional investigations, since it carries out most of the metabolic reactions involved in growth and development of higher animals in a self-enclosed system which is both readily obtained and readily handled. Results with it are not subject to many of the complications introduced in higher animals by possibilities of intestinal synthesis of food factors, etc. The above investigations indicate how it may be used in conjunction with metabolic inhibitors to approach many problems in a new and different manner.

HEME SYNTHESIS BY RED CELLS

The *in vitro* synthesis of heme, the red pigment of blood, by immature red cells has been demonstrated by a group of workers (R. J. Walsh and co-workers, *Science* **110,** *396 (1949)*) at the Harvard Medical School and Massachusetts Institute of Technology using radioiron as a tracer. Either Fe^{59} or Fe^{55} was added to the blood or bone marrow preparation contained in rocking vessels which were incubated at 37°C. in a gas atmosphere of 95 per cent oxygen to 5 per cent carbon dioxide. Incubations lasted from four to twenty-four hours and were conducted with sterile precautions in the case of periods longer than six hours. To determine the uptake of radioiron, the incubated blood was separated into a red cell fraction, and this was washed five times with ten times the fraction volume of 1 per cent saline.

The red cells of blood containing less than 1 per cent reticulocytes incorporated no measurable quantity of radioiron, and earlier

studies by other workers had shown that there is no uptake of radioiron by mature red cells from the plasma. In contrast, blood samples from cases of iron-deficiency anemia, hemolytic anemia, sickle cell anemia, and pernicious anemia, which may contain higher proportions of reticulocytes, show uptake of radioiron by the red cells. This uptake was a function of the reticulocytes present, and was readily demonstrated by fractionating the cells by specific gravity flotation and finding a direct correlation between the percentage of reticulocytes and radioiron content of the fractions.

It was also possible to demonstrate that the early reticulocytes resulting from liver therapy in pernicious anemia incorporate more radioiron than the later more mature reticulocytes. Thus in a case of pernicious anemia studied, the parallel relation between radioiron concentration of the incubated red cells and percentage of reticulocytes was demonstrated. In addition, however, a four- to fivefold greater radioactivity was found in the reticulocytes of the blood drawn one to three days after liver therapy than in the reticulocytes present five to ten days after initial liver therapy.

Of interest to the yet unsolved problem of the specific synthetic and structural deficiency in pernicious anemia was the investigators' analysis of the radioiron in the reticulocyte. The incorporation of radioiron represented, at least in part, a synthesis of hemoglobin in the reticulocyte since the recrystallized heme from these cells contained radioiron, but this fraction was termed only "significant" in contrast to the larger portion which was found in the stroma of the hemolyzed cells. This observation is in agreement or indeed the basis for the higher radioiron content of the early reticulocytes resulting from liver therapy of pernicious anemia, since these cells contain more reticular material.

Some studies were made on factors controlling the uptake of iron by the reticulocytes. Ferrous iron was assimilated more rapidly than ferric iron, and these more extensively than the iron from the "iron-binding" protein of the serum. Lack of glucose in the medium and low temperature decreased iron incorporation by the reticulocyte.

Bone marrow aspirations demonstrated that the normoblasts in such preparations incorporated radioiron even more rapidly than reticulocytes. Here again it was possible to show that the heme from these cells contained radioactive iron after incubation. Employing marrow cells from sternal aspiration of patients with untreated pernicious anemia it was possible to show that the *in vitro* addition of folic acid or liver extract caused an increased incorporation of radioiron. A 25 to 50 per cent increase in radioiron intake by these agents, in the case of pernicious anemia, was in contrast to the unchanged or slightly inhibitory effect of folic acid and liver on marrow cell samples from cases of malaria or iron-deficiency anemia.

These studies demonstrate that immature red cells are capable of heme synthesis even in the circulating blood, and that agents such as folic acid and liver extract can exert their effect on the immature but already formed erythrocyte.

INFLUENCE OF THIAMINE INTAKE ON PHYSIOLOGIC RESPONSES

The literature on thiamine requirements of man has been reviewed (*Nutrition Reviews* **6,** *319 (1948)*). Several studies are in agreement that the minimum thiamine requirement is 0.23 mg. or less per 1000 calories (A. Keys, A. F. Henschel, O. Mickelsen, and J. M. Brozek, *J. Nutrition* **26,** *399 (1943)*; Keys *et al.*, *Am. J. Physiol.* **144,** *5 (1945)*; M. K. Horwitt, E. Liebert, O. Kreisler, and P. Wittman, *National Research Council Bull.* **116** *(1948)*; V. A. Najjar and L. E. Holt, *J. Am. Med. Assn.* **123,** *683 (1943)*), but other researches

indicate higher values. Taking into account the need for a margin of safety, the National Research Council accepted the value of 0.5 mg. for each 1000 calories as a safe allowance for adults at ordinary low levels of caloric intake. This represents a workable goal of dietary planning. However, there is need for further information on physiologic responses of individuals maintained on varied levels of thiamine intake. This was the object of a study by W. W. Tuttle and associates (*J. Am. Dietet. Assn.* **24,** *945, 1049 (1948)*; **25,** *21, 123, 221, 322, 398 (1949)*) carried out in the Departments of Nutrition and Physiology, College of Medicine, State University of Iowa.

In the physiologic frame of reference, dietary requirements for a nutrient may be defined as that intake level of the nutrient which allows for preservation of a normal functioning of bodily processes dependent on the particular nutrient. When all the functions of a nutrient are not known or when we deal—as in the case of B-complex vitamins—with nutritional factors playing a profound role in the maintenance of bodily machinery, it is desirable to measure at least the principal components of man's total physiology. Bodily functions which should be considered in a comprehensive research on human nutrition were outlined by A. Keys (*J. Am. Dietet. Assn.* **21,** *211 (1945)*).

Research on nutritional requirements is in principle simple. The measurements of bodily functions are applied during the control period and repeated at intervals during subsistence at a given dietary regimen while a control group, maintained on a level which provides a level of intake considered as definitely safe, is tested under the same conditions as the experimental group. Significant alterations in the functions involved may then be related to the reduced level of the particular nutrient.

Though the principle is simple, comprehensive application of it is laborious and costly. The Iowa investigators' program was limited, i.e., it did not provide for the study of psychologic aspects proper and considered only limited aspects of neuromuscular performance. The study was reported in seven parts: I. Low-thiamine diets; II. Urinary excretion of thiamine; III. Reaction time; IV. Vision and hearing; V. Maximum work output; VI. Oxygen consumption; and VII. Thiamine requirements and their implications.

Healthy young women, 20 to 35 years of age, students at the University of Iowa, served as subjects. They lived together in a nurses' residence and ate the special diet in one of the dining rooms at the University Hospital, under the supervision of the Department of Nutrition. The research extended over two years. The first year (Experiment I) was devoted to the development of low-thiamine diets and of technics of measurement.

During the second year a new group of 12 subjects was observed for forty-five days of maintenance on a diet providing about 1200 micrograms of thiamine per day (control period). During the next six weeks, 6 subjects received daily about 140 micrograms of thiamine while 6 received in addition 1200 micrograms of thiamine (Experiment II). At the close of Experiment II all subjects were given large doses (not specified) of thiamine and ate adequate diets for seventeen days. The recovery from thiamine deficiency, taking place at this time, was not followed.

In Experiment III, 4 of the subjects were placed for nineteen weeks on a daily intake of 200 micrograms of thiamine, while 3 subjects who received 140 micrograms in Experiment II were now given a total of 1000 micrograms (200 + 800), and an intermediary group of 3 subjects subsisted on 625 micrograms (200 + 425). Two subjects withdrew from the experiment because of symptoms of appendicitis.

In the first communication, menus were given for three meals per day for the two basal diets providing approximately 140 and 200 micrograms of thiamine. The diet contained a normal amount of protein (55 to 60

g. per day) and provided daily about 2500 calories. The intake was adjusted to meet the individual caloric requirements by changing the servings of energy-giving food, low in thiamine. The diet was considered palatable and was eaten for extended periods of time without protest from the subjects. A list of foods used in the diet was included, together with their calculated and assayed thiamine values, and actual recipes were given. This is a welcome addition to the meager information on preparation of low-thiamine diets.

In Experiment II the subjects on restricted thiamine intake excreted, on the average, 19.3 micrograms per day during the first week and 17.8 micrograms during the sixth week. The change of −1.5 micrograms was statistically not significant. No excretion levels for the preceding forty-five-day "control" period were given. During the first five weeks of Experiment II the 6 supplemented subjects (+1200 micrograms of thiamine) excreted on the average 19.2, 36.4, 70.2, 203.2, and 243.7 micrograms per day. The data for the sixth week are not complete, but in the subjects for whom the data were available there was a further rise of 40.0 micrograms.

In Experiment III, following shortly after Experiment II, in 4 subjects whose average daily thiamine intake at the sixth week of Experiment II was 1338 micrograms and the excretion 281 micrograms per day, on 200 micrograms intake the average excretion values for five consecutive months were 32, 18, 16, 10, and 8 micrograms. In 3 subjects, unsupplemented in Experiment II (sixth week's intake 133 micrograms, excretion 18 micrograms), placed on an intake of 1000 micrograms in Experiment III increased their excretion from 47 micrograms in the first month to 71 micrograms in the fifth month. In subjects on 625 micrograms intake there was only a moderate rise in excretion (from 36 to 43 micrograms). Marked individual differences in excretion levels of the subjects maintained on identical intake levels were noted. On the intake of 140 + 1200 micrograms, subject #7 excreted during the sixth week of Experiment II only 160 micrograms, while subject #11 excreted 396 micrograms (ratio of 1:2.5). The individual variations in excretion were much greater on the high- than on the low-intake levels.

Anorexia was not evidenced by any subject on any level of thiamine used in these experiments and the body weight remained constant within ±2 pounds throughout the entire observation period.

Simple reaction-time tests were used in both Experiments II and III. A light served as a stimulus, while pressing a switch constituted the response. Two conclusions are fairly definitive. In Experiment II the maintenance on restricted intake resulted over six weeks in a significant slowing down of the response. In Experiment III the high and intermediate intakes were sufficient for maintenance of normal reaction times during five months of the experiment. On the low intake some subjects "deteriorated" while others "improved." The average values in the 4 subjects showed no change.

In Experiment II a comprehensive ophthalmologic examination was made, covering anatomic aspects, refraction, and visual fields. Also, the acuity of hearing in both ears was measured at 64 to 8192 cycles per second. The examinations were made at the beginning of the control period and at the end of the experimental period. No significant changes were observed.

In a bicycle-ergometer test of maximal work output the subjects did not reach a training plateau during the control period and continued to improve during the experimental period of six weeks (Experiment II). There was no significant difference between the improvement of the control and the experimental group. In Experiment III, extending over nineteen weeks, there was a marked decrement in work output in the group receiving the basal diet which contained 200 micrograms of thiamine, some decrement in the group on 625 micrograms of thiamine, and no change in the group on the

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intake of 1000 micrograms of thiamine. In all considerations involving nutritional deficiencies both the intake level and the time, as well as the general caloric level, must be taken into account.

To test the mechanical efficiency in physical work of moderate intensity, the subjects were required to pedal on a bicycle ergometer at a uniform rate. Oxygen consumption during one minute of work and ten minutes of recovery was measured. In Experiment II the experimental subjects became less efficient while the control subjects somewhat improved their efficiency. In Experiment III the same phenomenon was observed.

The literature on thiamine studies in man was briefly reviewed and the implications of the findings for the problem of thiamine requirements were discussed in the final paper of the series. The authors place the minimal daily thiamine requirement of young adults (women, normal body weight, intake of 2500 calories of mixed diet) at 0.25 to 0.30 mg. per 1000 calories, but point out that this represents a value applicable to the majority, but not to all subjects.

The critical level of most any nutrient will depend in a significant measure on several factors, such as the duration of the diet and the previous dietary status. One of the important factors determining the requirement level of nutrients is the criterion of fitness, and fitness in itself is difficult to measure. In the studies reported in this review, maximum work output and mechanical efficiency were the physiologic responses most sensitive to differential thiamine intake.

METABOLISM OF COENZYMES I AND II

With the advent of the isotopic tracer technic, all cell constituents, even those associated with less active parts of the cell, have been shown to undergo constant synthesis and degradation during the life of the cell. This constant synthesis and degradation of cell compounds is frequently referred to as "turnover." While the rate of turnover for different compounds seems to vary widely, the principle of turnover as a fundamental characteristic of metabolism is now unquestioned. Although a large amount of information has been obtained about the fate of substrate in the cell, comparatively little is known about the metabolism of the vitamins and coenzymes as well as other biocatalysts present in low concentrations. That many of the vitamins form coenzymes and thus exercise their biologic potency is well established, but the mode of coenzyme synthesis and degradation, the nature of the enzymes responsible, and the effect of activity in an enzymatic system upon the rate of turnover of its specific coenzyme are questions which, until recently, have remained largely unanswered.

The pyridine nucleotides (diphospho- and triphosphopyridine nucleotide) are two coenzymes which have been known for a comparatively long time, are required for a variety of enzyme reactions, and are present (particularly DPN) in reasonably high concentrations in the cell. Coenzyme I (DPN) is a dinucleotide composed of the B-complex vitamin niacin in the form of nicotinamide mononucleotide and adenylic acid. Coenzyme II (TPN) is a similar dinucleotide containing an additional mole of phosphoric acid. The two coenzymes are interconvertible by reversible enzymatic phosphorylation as shown by E. Adler, S. Elliot, and L. Elliot (*Enzymologia* **8,** *80 (1940)*), and although the position of the third phosphate moiety is still unsettled, the bulk of the evidence indicates that it is bound to the third carbon of the adenylic acid ribose (F. Schlenk, "A Symposium on Respiratory Enzymes," *p. 104, The University of Wisconsin Press (1942)*).

The synthesis of nicotinamide mononucleotide is apparently the first step in the synthesis of DPN. The vitamin niacin is required for this synthesis by most organisms, although certain lower bacteria and molds can build it up from carbon dioxide and ammonium while many mammals synthesize it, at least in part, from the amino acid tryptophan (*Nutrition Reviews* **7,** *307* (*1949*)). Certain hemophilus organisms require nicotinamide riboside (V factor) preformed in order to complete the synthesis of DPN. In certain bacteria (*Ibid.* **7,** *133* (*1949*)) and in red blood cells (P. Handler and H. I. Kohn, *J. Biol. Chem.* **150,** *447* (*1943*)) niacin is a better precursor of DPN than nicotinamide, suggesting that niacin riboside is formed prior to amidation.

The studies of A. Kornberg (*Abstracts, Am. Chem. Soc., 116th Meeting, 47C* (*1949*)) indicate that synthesis of DPN from nicotinamide mononucleotide is achieved through the action of adenosinetriphosphate (ATP). From autolysates of ale yeast and from extracts of hog liver acetone powder, this worker has isolated an enzyme which catalyzes the following reaction:

Nicotinamide mononucleotide + ATP ⇆ DPN + inorganic pyrophosphate. This requirement for ATP in DPN synthesis explains results of previous investigators who observed that substrate addition to yeast increased cozymase content while the addition of inhibitors of glycolysis or respiration invariably depressed the coenzyme levels. Kornberg has also purified an enzyme from autolyzed ale yeast which catalyzes the phosphorylation of DPN to TPN by ATP.

Since the above reactions are reversible, decomposition of TPN and DPN to their respective reactants is one possible mode of pyridine nucleotide degradation. Pyrophosphatases from washed kidney and potato, however, have also been shown by A. Kornberg (*J. Biol. Chem.* **174,** *1051* (*1948*)) to decompose DPN with the formation of nicotinamide mononucleotide and adenylic acid. The products of the activity of purified pyrophosphatase upon TPN are nicotinamide mononucleotide and a product containing adenine and phosphorus in a ratio of 1:2 which appears to be adenosine-3,5-diphosphate.

In minced brain, liver, and kidney tissue, however, another mode of degradation of DPN exists. P. Handler and J. R. Klein (*J. Biol. Chem.* **143,** *49* (*1942*)) showed that inactivation of DPN was accompanied by equimolar liberation of nicotinamide without appearance of orthophosphate or adenylic acid. The earlier observation by P. J. G. Mann and J. H. Quastel (*Biochem. J.* **35,** *502* (*1941*)) that nicotinamide added in relatively large amounts to minced brain tissue inhibited the inactivation of coenzyme I, can thus be explained if one assumes a mass-law effect of added nicotinamide upon the rate of degradation of the coenzyme.

H. McIlwain and D. E. Hughes (*Biochem. J.* **43,** *60* (*1948*)) report that a similar type of decomposition of DPN occurs in washed suspensions of *hemolytic streptococci*, *E. coli*, *Proteus morganii*, and *Hemophilus parainfluenzae* but not in *Lactobacillus arabinosus*. Both synthesis of cozymase from added niacin plus adenylic acid, and the liberation of nicotinamide from added cozymase with resulting inactivation were observed to occur rapidly in *hemolytic streptococci*.

Since *L. arabinosus* behaved anomalously in the above tests, and because it is the organism commonly used in the microbiologic assay of niacin, further studies of cozymase metabolism in this organism were carried out by McIlwain and his colleagues (H. McIlwain, D. A. Stanley, and D. E. Hughes, *Biochem. J.* **44,** *153* (*1949*); McIlwain, *Proc. Roy. Soc.* (*London*) *B* **136,** *12* (*1949*)). These workers found that while washed suspensions of *L. arabinosus* held for six days in saline medium at 37° showed no loss of total niacin, a shift of niacin or its complexes (60 per cent assayable as V factor) from cells to medium occurred. In the presence of glucose, however, there was a decline in total niacin in the suspension with a much smaller shift

from cells to medium. By repeating these experiments with growing cells under the usual assay conditions it was again observed that the presence of glucose caused significant amounts of total niacin to disappear (greater than 90 per cent of all of the niacin in this organism is DPN). While no direct proportionality between lactic acid formation under these conditions and rate of growth of cells or total cell mass was observed, it did appear that the rate of acid production was directly related to rate of disappearance of niacin.

Since the destruction of niacin (cozymase) appeared to be dependent upon the activity of the coenzyme as a codehydrase, these workers have termed the ratio (moles of lactate formed/mole niacin lost) the catalytic capacity of niacin. While catalytic activity of niacin, defined by these authors as the number of moles of lactate formed per mole niacin initially present per hour, is the type of turnover number of greater interest to the investigator doing a microbiologic assay for the vitamin, it is pointed out that catalytic activity expresses a much less fundamental relation than the catalytic capacity of niacin. While the catalytic activity is subject to wide variation depending upon the state of the cells, i.e., whether washed resting cells or an actively growing culture, the period of incubation, and the quantity of vitamin initially present, the catalytic capacity was remarkably constant under all of these conditions. While the products of degradation of DPN were not identified in this study, it appears to have been established that irreversible loss of DPN from the glycolytic system is a necessary concomitant of enzymatic activity. A. Lwoff and M. Lwoff (*Proc. Roy. Soc.* (*London*) *B* **122,** *360* (*1937*)) previously observed a similar relation between the activity of DPN as a codehydrase in *Hemophilus influenzae* and its inactivation, suggesting that it might be possible for DPN to be reduced the "wrong way" in the course of its oxidation-reduction.

Although the mechanism of this inactivation of coenzyme as a consequence of its activity in an enzyme system is still unknown, it provides an explanation for the need of constant vitamin intake, and constitutes an exciting challenge to the biochemist in the field of coenzyme metabolism.

NERVOUS SYSTEM LESIONS IN THE RHESUS MONKEY DUE TO THIAMINE DEFICIENCY

The interesting studies of thiamine deficiency in the rhesus monkey by J. F. Rinehart and L. D. Greenberg, working with L. L. Ginzton and M. Friedman have been reviewed (*Nutrition Reviews* **6,** *110* (*1948*); **7,** *149* (*1949*)). Seven monkeys were fed a purified diet without thiamine, alternating with short periods in which small amounts of thiamine were added. A plateau of body weight occurred which was often followed by weight loss. These changes in body weight were associated with diminished food consumption. At this time there was a marked reduction in the blood thiamine concentration. Apathy and weakness were characteristic and, finally, ataxia, tremors, and often ptosis developed. Thiamine administration produced a dramatic response within twenty-four to forty-eight hours unless deprivation had been carried on too long. There was a slight but definite reduction in the red blood cell count, hemoglobin concentration, and the reticulocyte count. Following administration of thiamine there was a mild reticulocytosis but the red blood cell count and hemoglobin concentration failed to return to normal.

Central nervous system changes were thought to resemble those of Wernicke's disease except that the vascular lesions seen in this condition were uncommonly seen in the thiamine-deficient monkeys. There were rather marked changes in many of the cere-

bral nuclei and in the gray matter of the cerebral cortex.

More extensive pathologic analysis of the nervous system lesions in these monkeys has been reported by J. F. Rinehart, M. Friedman, and L. D. Greenberg (*Arch. Path.* **48,** *129* (*1949*)). The pathologic findings in the nervous system are described in some detail. In general, the changes were those of degeneration which were bilaterally symmetrical. The bilateral symmetry of the lesions suggested that vasospasm was an unlikely cause.

The location of the lesions in the gray matter was thought to be due possibly to the much greater metabolic activity of this tissue as compared with the white matter, which presumably has a lower metabolic activity and somewhat greater vascularity. Vascular changes were seen in the brain but they were usually late manifestations and then only in moderate degree. In this regard the lesions differed somewhat from those of Wernicke's syndrome in man although the two were similar with regard to symmetry of the lesions and their distribution. The authors suggest that the vascular changes in Wernicke's syndrome in man may be the result of deficiencies of other nutrients although none is specifically incriminated.

Peripheral neuropathy was not found histologically in any of the 7 thiamine-deficient monkeys, nor was there any clinical evidence of a sensory neuritis. The lack of peripheral neuropathy in these animals adds further evidence to the view that thiamine is not the antineuritic vitamin (A. P. Meiklejohn, *New Engl. J. Med.* **223,** *265* (*1940*)). Other studies have indicated "that, if there are no great species differences, the neuropathologic syndrome previously attributed to chronic thiamine deficiency may actually be due in large part to a lack of other dietary essentials" (*Nutrition Reviews* **1,** *210* (*1943*)). The possibility that some of the peripheral nerve symptoms in man may be the result of a biochemical or physiologic lesion due to thiamine deficiency is suggested in a previous review (*Ibid.* **1,** *28* (*1943*)).

Deficiency of nutrients other than thiamine has been shown to be responsible for neurologic lesions in experimental animals (*Nutrition Reviews* **1,** *137* (*1943*)). These have included pyridoxine, pantothenic acid, niacin, and possibly riboflavin. Extensive, well-controlled investigations are still necessary before a clearer correlation may be made between the diverse neuropathologic lesions seen clinically (D. Denny-Brown, *Ibid.* **6,** *10* (*1948*)) and deficiency of specific nutrients.

STUDIES OF HUNGER EDEMA IN GERMANY

Investigations of hunger edema and its relation to serum protein concentration, particularly that of serum albumin, have led to doubt as to the applicability of the Starling hypothesis, in which the osmotic pressure of the plasma proteins is the primary factor in preventing edema, to all instances of edema (*Nutrition Reviews* **4,** *317* (*1946*); **5,** *31* (*1947*); **6,** *210* (*1948*)). Some reports from Germany have added data to the problem although there is little clarification of the pathogenesis of hunger edema.

G. Herrnring (*Klin. Wchnschr.* **26,** *296* (*1948*)) studied 535 cases of undernutrition seen in Hamburg between April, 1945 and March, 1946. He found a rough correlation between total serum protein concentration and appearance of edema, but observed that between 60 and 70 per cent of patients with serum protein concentrations above 6 g. also had edema. Although the author points out the necessity of measuring the individual plasma proteins, especially the serum albumin concentration, fractionation was done in this series in only 168 patients. Of these, 6 had a serum albumin concentration under 2.5 g. per 100 ml. and had edema. Severe edema seemed to disappear with serum al-

bumin concentration over 5 g. per 100 ml. In addition, the author remarked upon the occurrence of serum protein concentrations above normal in patients with high-grade undernutrition.

H. W. Bansi and G. Fuhrmann (*Klin. Wchnschr.* **26,** *326, 358 (1948)*) stressed the importance of the caloric content of the diet in the metabolism of protein, indicating that when the caloric content of the diet was restricted in Germany, during severe food rationing, to under 2000 calories per day, a considerable amount of protein was destroyed by catabolism of body tissue. These authors carried out studies of the relation between sulfur and nitrogen in the urine. They noted that the relation between nitrogen and sulfur was constant at between 14 and 16 to 1, not only in the food but also in body protein and in urine. The disturbance in this relation in hunger edema was studied. Evidence was presented that the sulfur was rebuilt, presumably into body tissue, somewhat more rapidly than nitrogen in patients with hunger edema.

Further observations relating to hunger edema were made by E. Klotzbücher (*Klin. Wchnschr.* **26,** *289 (1948)*). This author noted, in many patients, the fall in plasma protein, preferentially that of albumin, with an occasional rise in globulin and an increase in fibrinogen concentration. The severity and duration of the edema appeared to depend upon the concentration and the composition of the plasma proteins. When edema disappeared, the plasma protein concentration rose, largely due to an increase in albumin, even though there was a fall in globulin to some extent. The circulating red cell mass was smaller than normal during the height of the edema and rose as edema disappeared. In addition, in the edematous patient an increase in heart size and changes in the electrocardiogram characterized by flattening of T waves without change in the QT interval were observed. These changes were noted to be reversible. Other observations included a decreased rapidity of disappearance of subcutaneous injections of normal saline, a fall in blood pressure, a decreased content of enzyme and hydrochloric acid in the gastric juice, and in some instances a blood sugar curve after the oral administration of carbohydrate similar to that found in diabetes.

Many of these observations confirm the results of others. It may be suggested that careful clinical and metabolic studies are badly needed to further knowledge of the physiologic basis of hunger edema. Investigations of cardiovascular and renal function are particularly required. Although it is hoped that hunger edema will soon disappear, future studies, if they are made, should be carefully planned so that the maximum information can be obtained.

ASCORBIC ACID AND ADRENAL FUNCTION

Ascorbic acid is known to be related intimately to the functional activity of the adrenal cortex, although the exact biochemical mechanisms are still obscure. Evidence has been presented indicating that ascorbic acid is necessary for production of cortical hormones (*Nutrition Reviews* **3,** *37 (1945)*). If stimuli are applied to cause increased production of cortical hormones, the ascorbic acid concentration in this gland drops quickly. If these stimuli are continued over a sufficient period, the adrenal gland enlarges and the ascorbic acid concentration is increased rather than decreased (H. Selye, *Endocrinology* **21,** *169 (1937)*; D. J. Ingle, *Am. J. Physiol.* **124,** *369, 627 (1938)*; *Endocrinology* **24,** *194 (1939)*; *Nutrition Reviews* **6,** *143 (1948)*). Some evidence has been presented suggesting that ascorbic acid may be synthesized in the adrenal (*Ibid.* **8,** *25 (1950)*).

The stimulus for increased cortical ac-

tivity may be varied. Apparently almost any type of stress will produce this reaction, or it may be produced physiologically using adrenocorticotropic hormone. Augmented cortical activity is apparently one of the mechanisms by which an animal adapts to a cold environment. L. P. Dugal and M. Thérien (*Nutrition Reviews* **6,** *143 (1948)*) have reported that rats and guinea pigs, after long exposure to cold, showed an increase in adrenal ascorbic acid when adaptation was achieved. If the animals failed to adapt, the contrary occurred, namely, a decrease in adrenal ascorbic acid. There was also a relation between the dietary supply of ascorbic acid and ability to adapt to the cold.

L. P. Dugal and M. Thérien (*Endocrinology* **44,** *420 (1949)*) have continued their studies to determine whether relatively large doses of ascorbic acid would have any effect on the adrenal hypertrophy normally observed during exposure to cold. Both rats, which normally synthesize their requirement for ascorbic acid, and guinea pigs, which require exogenous ascorbic acid, were used in their experiments.

One group of rats was kept in an environmental temperature of −1°C. and another group was kept as controls at room temperature. Half of each group received 150 mg. of ascorbic acid, as sodium ascorbate, intraperitoneally each day, the controls receiving an equivalent amount of saline. At the end of three days, the body, adrenal, and kidney weights were determined. As expected, the adrenals of the nonsupplemented animals kept in the cold were enlarged compared with their room-temperature controls. The unexpected finding was that adrenals of the ascorbic acid-treated rats kept in the cold were not enlarged. A similar phenomenon was noted with kidney weights. Ascorbic acid also had a markedly favorable action on survival in the cold. When the experiment was continued for a total of seven days, 75 per cent of the animals receiving ascorbic acid were surviving, whereas none of the nonsupplemented rats was living.

A similar experiment was conducted with rats over a period of two hundred and forty-four days using an environmental temperature of 4°C. The same phenomena were observed, the effect of ascorbic acid being even more striking than in the first experiment. The adrenals of the ascorbic acid-treated rats in the cold were essentially the same as the room-temperature controls while those in the cold without ascorbic acid showed a 22 per cent enlargement. It should be mentioned that the adrenal weights were reported and compared on a wet-weight basis. Because of the known effect of some of the cortical hormones in altering water and electrolyte balance in the tissues, it is possible that some of the enlargement may have been more apparent than real. Eighty per cent of the ascorbic acid-treated rats survived in this experiment as compared with only 51.4 per cent of the untreated rats. Ascorbic acid also promoted somewhat better growth in the rats kept in the cold. This effect was not seen at room temperature. It is of some interest to note, although the authors do not mention it, that ascorbic acid under these conditions behaves like a vitamin essential for the rat in that it promotes growth, improves survival, and influences tissue reactions.

Guinea pigs were used in a similar type of experiment. Although the conditions were somewhat different, the same type of results was obtained in that ascorbic acid promoted growth and almost completely prevented adrenal enlargement, effects which were not noted at room temperature. More ascorbic acid was retained in the adrenals of the ascorbic acid-treated animals in the cold room than at room temperature. Amounts of ascorbic acid which are generally considered to be barely antiscorbutic (2 mg. daily by mouth) would not prevent the adrenal enlargement, although 25 mg. daily would.

These experiments demonstrate another interesting aspect of the ascorbic acid-adrenal relation and will undoubtedly provide a basis for much further experimentation.

They also provide a stimulus for some interesting speculation as to the function of ascorbic acid. The fact that this vitamin prevents adrenal enlargement under these conditions might mean that in some manner it can substitute for the cortical hormones in so far as adaptation to cold is concerned. This possibility is strengthened, superficially at least, by the fact that if sufficient cortical extract is given to animals under stress, adrenal enlargement likewise will be prevented. If it is assumed that ascorbic acid is synthesized in the adrenal gland, for which there is inconclusive evidence, and since the animal adapting to the cold needs more ascorbic acid, it is possible that adrenal enlargement is primarily a response enabling the gland to produce more of this vitamin. Then there is the possibility that ascorbic acid is used up in the production of cortical hormones. Thus the rat, which normally synthesizes enough ascorbic acid, would need supplementation under these conditions, and the guinea pig, which normally synthesizes an insufficient amount, if any, would need increased amounts.

Lastly, there is the possibility that the biochemical mechanisms necessary for production of cortical hormones simply crowd out ascorbic acid until adaptation has occurred at which time the ascorbic acid concentration returns to a new normal level in proportion to the heightened metabolic activity of the tissue. This assumption would not explain why ascorbic acid prevents adrenal enlargement. Many facts and arguments could be marshaled for and against each of these possibilities, and other possibilities could be offered. The true explanation may involve several of these factors. Only further experimentation will provide definite answers.

These studies also raise a question as to the role of the thyroid in this process. Altered thyroid activity is known to occur with changes in environmental temperature. Experimental hypothyroidism tends to produce a decrease in adrenal size (C. P. Leblond and H. E. Hoff, *Endocrinology* **35,** *229* (*1944*); E. J. Baumann and D. Marine, *Ibid.* **36,** *400* (*1945*)), while induced hyperthyroidism is accompanied by an increase in adrenal weight (H. W. Deane and R. O. Greep, *Ibid.* **41,** *243* (*1947*)).

D. P. Wallach and E. P. Reineke (*Endocrinology* **45,** *75* (*1949*)) have presented additional studies in this direction. By administering various amounts of thyroxine to rats for varying periods of time, they were able to demonstrate an increase in adrenal weight and adrenal ascorbic acid concentration if the thyroxine dosage was sufficient and the administration continued long enough. The immediate effect of thyroxine was to depress adrenal ascorbic acid similar to that shown with other types of adrenal stimulation. By injecting adrenocortical extract in rats receiving thyroxine, they were able to prevent the adrenal weight and ascorbic acid changes which would otherwise have occurred. Between 10 and 20 dog units of adrenocortical extract daily were required to produce this effect in rats receiving 20 micrograms of thyroxine per 100 g. of body weight daily.

This result would seem to strengthen the assumption that changes in adrenal weight and ascorbic acid occurring during thyroidal stimulation and other stresses do reflect changes in the output of adrenocortical hormones, although the possibility that adrenocortical extract might contain sufficient ascorbic acid to contribute to this result needs some attention. Whether the adrenal changes occurring during cold adaptation are mediated by the thyroid is not established by these studies but is a possibility which should be investigated.

KWASHIORKOR

Kwashiorkor, or malignant malnutrition, is a syndrome commonly observed in African children and characterized by impairment of growth, edema, lowered concentration of plasma albumin, steatorrhea, fatty infiltration of the liver, anemia, and a characteristic dermatitis frequently termed "crazy-pavement dermatosis." Depigmentation of the hair and skin may occur. The characteristics of the anemia vary and may at times be macrocytic; at other times, microcytic.

The accumulated evidence indicates that this syndrome is at least in part due to malnutrition. To date, however, it has not been possible to link the development of the disease to a deficiency of one specific nutrient (*Nutrition Reviews* **4,** *303* (*1946*); **5,** *353* (*1947*); **6,** *270* (*1948*)).

H. C. Trowell (*Tr. Roy. Soc. Trop. Med. Hyg.* **42,** *417* (*1949*)) has again described the disease and reflected on its significance from the vantage point of one with twenty years of association with the syndrome as observed in Uganda and Kenya. He emphasizes that the most significant dietary defect appears to be the shortage of first-class protein of animal origin. Further, it is noted that the "nutritional anemia" characteristic of the disease is not accompanied by megaloblastic marrow and fails to respond to folic acid. In addition, Trowell finds that the syndrome observed in Uganda, in contrast to that seen in South Africa and termed "infantile pellagra," does not respond clinically to desiccated stomach. The syndrome differs in some details from the fatty liver disease of infants as studied by J. C. Waterlow (*Med. Res. Council, London, Special Report Series* **263** (*1948*)).

In keeping with much modern nutrition thinking, Trowell expresses the opinion that continued presence of the chronically malnourished state which is responsible for Kwashiorkor may lead to irreparable tissue changes, impairment of health, premature death, and senility.

This interesting review is concluded with a valuable bibliography of one hundred and twenty-five references.

RIBOFLAVIN IN DEVELOPING EGGS OF THE GRASSHOPPER

From the study of riboflavin metabolism of the developing egg of the grasshopper, *Melanoplus differentialis*, has arisen the most provocative hypothesis that riboflavin may serve as a precursor of pterin pigments. Evidence in support of this hypothesis is still inconclusive but some of the most important evidence may be reviewed.

Two pterins, xanthopterin and leucopterin, have been identified as pigments occurring in insects (F. G. Hopkins, *Proc. Roy. Soc.* (*London*) *B* **130,** *359* (*1942*)). Pteroylglutamic acid and its homologues are structurally similar vitamins which are necessary for the nutrition of many species. Examination of the chemical structure of these pterins and of riboflavin reveals that the skeletal arrangement of the two condensed nitrogen-containing rings of riboflavin is similar to that of the pterin grouping.

J. H. Bodine and L. R. Fitzgerald (*Physiol. Zool.* **20,** *146* (*1947*)) prepared a 0.25 N sulfuric acid extract of eggs of the grasshopper, *M. differentialis*, of known ages and stage of development. These extracts, adjusted to pH 4.5, were used for a fluorometric determination of riboflavin. The usual precautions for the direct chemical determination of this substance were observed, including oxidation of interfering substances by potassium permanganate and hydrogen peroxide, as well as reduction of riboflavin with sodium hydrosulfite and employment of internal standards. Separation of riboflavin by adsorption was not performed. Analyses were made on a series of eggs, all from the same pod and, hence, from the same female,

as well as on different lots of eggs from assorted females but at the same developmental ages. Variations in results were of equal magnitude whichever procedure was adopted.

Throughout the developmental period of the egg, the total fluorescence at pH 4.5 remained essentially constant at the level of fluorescence which would be equivalent to about 0.08 microgram of riboflavin per egg. An unchanging quantity of this fluorescence, however, was due to a nonreducible material and, accordingly, the actual riboflavin content per egg approximated 0.06 microgram.

Embryos of different ages were dissected from their respective egg yolk, and separate determinations were made on the embryo and the yolk. As the embryo aged, its riboflavin content increased from 0.015 to about 0.045 microgram, while the yolk exhibited a decrease in its riboflavin content. Inasmuch as the riboflavin procedure employed was a direct chemical method and did not separate the riboflavin by an adsorption step, all of these measurements should be interpreted as representing riboflavin equivalents or, as the authors state, "riboflavin or its reducible derivatives."

Riboflavin does not fluoresce in the alkaline range of pH 10 to 11. Accordingly, the sulfuric acid extracts were made alkaline with potassium hydroxide and the fluorescence measured at pH 10 to 11. In the early developmental stage of the egg, a constant, but small amount of alkaline-fluorescing substance was found. This could not be reduced with sodium hydrosulfite. Further, this material proved to be nondiffusible.

In the later stages of the developing egg (postdiapause) two simultaneous changes occurred: (1) There was an increase in the fluorescence in the alkaline range of pH 10 to 11. This fluorescence was due to a diffusible substance which also fluoresced at pH 4.5 and which was reducible with sodium hydrosulfite in an acid medium. (2) Simultaneously there occurred a decrease in the fluorescence at pH 4.5 which was attributable to the loss of riboflavin. This loss of riboflavin continued until in the newly-hatched nymph there could be found no free riboflavin.

The appearance of a soluble pink pigment was noted simultaneously with the increase in alkaline fluorescence. It was suggested that this new pigment was a pterin which was derived from riboflavin.

These same workers (J. H. Bodine and L. R. Fitzgerald, *Physiol. Zool.* **21,** *93 (1948)*) extended their study to eggs in which the embryo was destroyed by x-irradiation. In such eggs, the embryo fails to develop, although the extraembryonic structures, such as the serosa and yolk, are left intact. In irradiated eggs, little, if any, change occurred in their riboflavin content and none of the pterin-like pigment appeared. The authors concluded that conversion of riboflavin into pterins appears to be a specific property of the embryo and is not associated with the extraembryonic structures.

L. E. Burgess (*Arch. Biochem.* **20,** *347 (1949)*) has made a preliminary study of these pigments which arise in the developing egg of the grasshopper. Eggs in lots of 500 were ground and successively extracted with ether, alcohol, and water. Two light-brown crystalline fractions and one purplish-brown fraction were subsequently isolated from these extracts. From a study of the physical properties, particularly the ultraviolet absorption curve and the fluorescence characteristics, it was concluded that these substances are most likely pterins and that they account for the alkaline fluorescent property which appears late in the development of the egg of the grasshopper.

This report does not contain evidences of the purity or positive identification of these crystals. It does, however, lend additional support to the hypothesis set forth by Bodine and Fitzgerald that riboflavin may serve as a precursor of pterins in the grasshopper. As such, the report should serve to stimulate a more extensive investigation of the chemistry of these compounds and the relation of

the appearance of the pigments to the disappearance of riboflavin in the developing egg of this species. It is possible that this is a fundamental relation.

HYPOCALCEMIA OF THE NEWBORN

During the first few days of life, changes occur in concentrations of calcium and phosphorus in the serum of normal infants. Characteristically there is a transitory decrease in the level of calcium and a rise in the concentration of phosphorus in serum (H. Bakwin, *Am. J. Dis. Child.* **54,** *1211 (1937)*). It has been suggested that these changes may relate to the occurrence of neonatal tetany, a syndrome characterized by the appearance of convulsions, vomiting, and edema in newborn children. Manifestations are frequently associated with a high-phosphorus and a low-calcium content of serum.

K. Dodd and S. Rapoport (*Am. J. Dis. Child.* **78,** *537 (1949)*) have published results of experiments evaluating the possible significance of hypocalcemia in determining the occurrence of clinical signs of neonatal tetany among infants admitted to Children's Hospital in Cincinnati. The diagnosis was made in but 5 infants admitted to this hospital during the seven-year interval from 1937 to 1944. With increased awareness of the syndrome, 12 infants with neonatal tetany were admitted during each of the years 1946 and 1947. A total of 33 cases of tetany of the newborn was recognized in the ten-year interval from 1937 to 1947.

Of the 33 infants, 20 were males. Birth histories and birth weights were normal in nearly all of the group. Clinical signs of neonatal tetany were noted during the first two days of life in 16 patients, and as late as the thirteenth day in but one infant. Manifestations of the disease were varied. Vomiting occurred in 28 infants, and in certain of the subjects was severe; convulsions were observed in 22. Sixteen patients were found to be edematous. Hemorrhagic manifestations occurred frequently. Although the clinical picture at times indicated consideration of traumatic lesions of the central nervous system and of congenital anomalies of the gastrointestinal tract as causative factors, there were no evidences of residual defects after recovery from manifestations of tetany.

Determination of the concentration of calcium in serum gave values of less than 9 mg. per 100 ml. in each of the 33 patients and less than 7 mg. per 100 ml. in 9 patients. In contrast, only 5 of 48 normal newborn infants were found to have concentrations less than 9 mg. per 100 ml. The phosphorus content of serum was above 8 mg. per 100 ml. in 10 of 25 infants with neonatal tetany, while this relatively high concentration was found in only 2 of 47 normal newborn infants. Low levels of calcium were not always associated with increase of serum phosphorus. Determination of serum alkaline phosphatase, nonprotein nitrogen, and total protein revealed no consistent or significant changes in concentration of these substances.

In an effort to determine more adequately the clinical manifestations likely to be associated with hypocalcemia the observations were extended. Each full-term newborn infant admitted to the nursery of the Cincinnati General Hospital was examined and observed. Serum specimens were obtained from each for determination of calcium, phosphorus, and nonprotein nitrogen. The earlier investigation had indicated a wide variety of possible manifestations of hypocalcemia. Therefore, a variety of clinical signs and symptoms was searched for in the group of apparently normal infants in the nursery. Among these were disturbances in respiration or of feeding, vomiting, edema, drowsiness, stiffness, irritability, convulsions, crowing, and ankle clonus. Appearance or persistence of any of these signs was taken as possible evidence of tetany. By this means the normal newborn infants were divided

into two groups, one a control group of 48 infants without the signs listed above, and the other a group of 24 infants with these probable manifestations of tetany. Two other groups were also studied.

There was a group of 9 seriously ill infants with clinically manifest tetany. Also included was a group of 12 premature infants with signs of tetany. Results of laboratory analyses done on these four groups of infants were compared. Concentrations of serum calcium in the 24 full-term infants with mild signs of tetany indicated lower calcium levels than in the control group of 48 infants. Two thirds of the group with evidences of tetany in mild form had concentrations of calcium less than 9 mg. per 100 ml., while values of this low level were observed in but one tenth of the control group. Still lower calcium values were found in the full-term infants with severe tetany and in the premature infants with manifestations of tetany. Differences were not so striking when serum phosphorus concentrations in the four groups were compared. However, among full-term infants there seemed to be a relation between the presence and severity of manifestations of tetany and the occurrence of phosphorus concentrations greater than 8 mg. per 100 ml. High levels of serum phosphorus were not observed in premature infants with signs of tetany.

The data presented indicate that there is parallelism between the occurrence and severity of symptoms of neonatal tetany and a decrease in concentrations of serum calcium. Although tetany occurred with relatively small decreases in calcium levels, the diagnosis is less likely if the calcium content of serum is greater than 9 mg. per 100 ml. It was also shown that the concentration of serum phosphorus in normal newborn infants is seldom greater than 8 mg. per 100 ml. One third of the infants with manifestations of tetany were found to have serum phosphorus values greater than 8 mg. per 100 ml. An increase in the phosphorus content of serum may represent important additional information confirming the diagnosis of tetany. The phosphatase activity of serum was normal in all of the infants with neonatal tetany, a finding which would seem to differentiate it from the tetany of rickets which is associated with high-alkaline phosphatase activity of serum.

Dodd and Rapoport conclude that tetany associated with hypocalcemia may occur in newborn infants and that it may be associated not only with neurologic manifestations such as convulsions, muscular rigidity, irritability, and lethargy but also with vomiting, cyanosis, edema, and hemorrhagic phenomena. Certain of the cases are associated with changes in electrolyte distribution resulting from vomiting, hyperventilation, and renal disturbances. A large group of cases remains, however, in which such causes have no demonstrable role. It is held that these may result from parathyroid insufficiency of the newborn. Although there is no evidence to indicate histologic immaturity of the parathyroid glands at the time of birth, it is wholly possible that the functional activity of the gland of the infant *in utero* is sometimes suppressed by hypertrophy of the parathyroid glands of the mother during pregnancy. In the interval immediately following birth parathyroid deficiency and hypocalcemia may become manifest.

Recognition of hypocalcemic tetany in the newborn is important since it can be adequately treated by administration of calcium salts. Dodd and Rapoport believe that dihydrotachysterol given in addition to calcium salts has an important adjuvant effect.

Infantile rickets does not usually become clinically manifest until after the first months of life. For this reason, and because alkaline phosphatase activity of serum is elevated in rickets, it has been thought unlikely that deficiency of vitamin D may be a causative factor in tetany of the newborn.

S. Ranström and G. von Sydow (*Pediatrics* **4**, *406 (1949)*) have, however, found morphologic evidences of rickets in the costochondral junctions of 29 per cent of 69 infants dying from various causes during the first day of life. Such evidence was found in

about the same proportion of premature as full-term infants. These observations indicate that congenital rickets occurs commonly. The possibility would remain that rickets present at birth might, also, account for certain cases of neonatal tetany.

It is unlikely that the diet of the infant influences the occurrence of neonatal tetany in an important manner, since the condition occurs frequently during the first forty-eight hours of life. It would be pertinent to investigate the possible effects of maternal dietary patterns on the occurrence of this disorder in infants.

PHYTIC ACID, VITAMIN D, AND ABSORPTION OF CALCIUM

Relatively early in the modern period of nutrition research it was demonstrated that inclusion of cereals in diets of growing animals hastened the occurrence and increased the severity of manifestations of rickets. Later this effect was ascribed to the presence in grains of relatively large quantities of phytic acid, the hexaphosphoric acid ester of inositol (*Nutrition Reviews* **3,** *61 (1945)*). It was demonstrated that phytate decreased the absorption of calcium from the intestinal tract of animals on diets inadequate in vitamin D. The concept was held that this mechanism was of little significance when adequate quantities of vitamin D were provided.

Sir Edward Mellanby, whose contributions to the understanding of the action of phytate have been of great importance and have covered a period of more than three decades, has extended his observations pertaining to mechanisms whereby phytate exercises its anticalcifying action and has demonstrated in puppies that this may occur even when vitamin D is provided (*J. Physiol.* **109,** *488 (1949)*).

Puppies used in these experiments were from laboratory-bred animals fed adequate quantities of calcium, phosphorus, and vitamin D during pregnancy and lactation. All puppies were placed on pre-experimental diets which were either supplemented with vitamin D so that reserves of the vitamin would be large or were not supplemented so that animals with low reserves would be provided. The calcium content of the basal diets was low but sufficient, with addition of vitamin D, to effect good calcification of bony structures. In each experimental group, comparable animals received the same amounts and ratio of calcium and phosphorus.

Oats or oatmeal containing large quantities of phytate were fed to puppies with high or low reserves of vitamin D, and bone growth and calcium absorption determined. Litter mates of these animals were fed oats in which the phytate phosphorus had been split to phosphate phosphorus by acid hydrolysis or by germination and autolysis. In each instance the diet containing untreated oats demonstrated a more marked effect in reducing calcium absorption than did the diets containing treated oats. Determination of the mineral ash content of bone and search for roentgenographic evidence of rickets showed more marked abnormalities when phytate was fed to animals with either high or low reserves of vitamin D. The quantity of vitamin D provided was not sufficient to prevent rickets when untreated oats were fed.

In other experiments, the sodium salt of phytic acid was prepared and fed instead of providing phytate in cereals containing other unknown nutritional factors. Again it was demonstrated that when vitamin D is present in the body, although none is contained in the food, phytate prevents the absorption and retention of calcium to a greater extent than phosphate containing equivalent quantities of phosphorus. If, however, vitamin D reserves of the growing animals were depleted, both phosphate and phytate greatly reduced calcium absorption, but the latter usually proved somewhat more effective even under these conditions.

When phytate was increased, replacing

phosphate in the diet of animals given vitamin D, two effects were observed. First, the amount of phytate excreted in the feces increased as larger quantities were fed. Evidence was also obtained that a varying proportion of the phytate in the diet cannot be accounted for by excretion in the urine and feces and that this quantity is increased as more vitamin D is added to the diet. There would seem to be a critical level of vitamin D at which maximal diminution in phytate excretion occurs.

Addition of phytate to the diet increased calcium excretion in the feces. Regularly, an increase in fecal phytate was associated with increased excretion of calcium. When the vitamin D in the basal diet was just sufficient to prevent rickets, then addition of phytate resulted in frank rickets. When supplies of vitamin D were greater, addition of phytate to the diet tended to produce osteoporosis of bone but not actual rickets. With diets poor in calcium and vitamin D, phytate may actually cause loss of calcium from the body, through mobilization of calcium from bone and excretion through the intestinal tract. This effect was only partially ameliorated by administration of vitamin D but was totally corrected by supplying additional quantities of both vitamin D and calcium.

If the diet of growing animals is relatively rich in phytate, several considerations are important in providing for optimum formation of bone. Calcium absorption must be sufficient to allow for incorporation of the mineral in bone and other tissues. The supply of vitamin D must be adequate, but of equal importance is provision of increased quantities of calcium in the diet.

Mellanby demonstrated a synergism between calcium salts and the vitamin. A puppy receiving only 5 I.U. of vitamin D_2 plus a dietary supplement of 200 mg. of calcium daily absorbed an amount of calcium equal to that of another animal receiving twenty times the quantity of vitamin D_2 but without the added calcium. It was also shown that calcium immobilization, caused by precipitation of insoluble pentacalcium phytate in the intestine, can best be overcome, if supplies of vitamin D are available, by increasing calcium intake.

Mellanby has demonstrated in these experiments that in dogs, phytate as compared with phosphate specifically reduces absorption of calcium, with most marked effect in the presence of vitamin D. Diets having the same amounts and ratio of calcium and phosphorus may have different effects on absorption of calcium and calcification of bone, depending upon the chemical nature of the phosphorus-containing substances in the diet. The amounts of dietary calcium and phosphorus necessary to produce optimal bone calcification vary greatly depending on other constituents of the diet. Of greatest importance is the content of vitamin D. The action of the vitamin can be enhanced by supplying additional calcium and decreased by adding phytate.

The over-all effect of phytate is that of an antagonist of vitamin D. Since there is interference with the action of the vitamin, but not by reason of chemical analogy, Mellanby considers phytate a toxamin rather that an antivitamin.

Mellanby points out that the observations made in dogs probably pertain directly to considerations relative to the effect of phytate on the calcification of bones in children. Evidence is available in a number of reports indicating that phytate interferes with the absorption of calcium from the intestinal tract of human beings (R. A. McCance and E. M. Widdowson, *J. Physiol.* **101,** *44 (1942)*; E. Hoff-Jørgensen, O. Andersen, and G. Nielsen, *Biochem. J.* **40,** *555 (1946)*). Administration of supplements of vitamin D did not increase calcium absorption in these studies.

The observations of Mellanby would seem to indicate that such an effect would not be anticipated because the quantity of vitamin D required to promote maximum absorption of calcium is small, and in man might be supplied from body reserves for prolonged periods of time. Only if stores of

the vitamin had been depleted would increased calcium absorption be expected to follow administration of supplements. In planning for favorable conditions for calcification of bones and teeth, the important consideration would seem to be to provide calcium not in quantities just sufficient to prevent negative balance but great enough to assure maximum absorption and retention.

ARTERIOSCLEROSIS IN RELATION TO FAT METABOLISM

Heart disease, with arteriosclerosis as a major contributory cause, presents a real challenge to the nutritionist and biochemist since many features in the nature and genesis of the atheromatous lesion are already recognized (*Nutrition Reviews* **6,** *99, 176 (1948)*; **7,** *155 (1949)*). Thus it is known that the arterial plaques themselves are rich in lipid materials, particularly in cholesterol and its esters, and that calcium becomes deposited at these sites. Atherosclerosis has been induced in rabbits by cholesterol feeding and in dogs by cholesterol and thiouracil feeding (*Ibid.* **6,** *255 (1948)*).

The association of arteriosclerosis with metabolic diseases characterized by high-serum lipid levels, and the marked correlation of obesity with atherosclerosis, are further evidences of the relation of fat metabolism to this form of degenerative disease. The cause of the initial change to the arterial wall which leads to cholesterol deposition is not well understood, although some investigators have presented evidence that macromolecular materials, such as the fat chylomicrons of the blood serum, can penetrate and damage the arterial wall, thus leading to development of arterial plaques.

In light of the foregoing, the report of G. H. Becker, J. Meyer, and H. Necheles (*Science* **110,** *529 (1949)*) is particularly pertinent in revealing a marked change of fat metabolism in the aged. These investigators have demonstrated that a high-fat meal results in a markedly greater number of serum chylomicrons and a more prolonged chylomicronemia in the aged than in the young human subject. Thirty young subjects (average age 18 years) and 30 old subjects (average age 76 years) were tested. The fat meal consisted of 0.5 g. of oleomargarine per kilogram of body weight, given with toast and tea. Finger blood was taken for a chylomicron count at intervals for as long as twenty-four hours if necessary.

The differences between the average curves for the two groups of subjects were striking. In the young age group the chylomicron count rose to a maximum of about 450 in three hours and returned to the base level in five hours. In contrast, the chylomicron count of the older individuals reached a maximum in eight to twelve hours and only returned to the base level in twenty-four hours. Such a time course of alimentary lipemia would mean that the older individual consuming the usual three meals daily might be in constant state of hyperlipemia. Since a blood level of any metabolite after feeding must represent a balance between absorption and utilization, it was desirable to determine whether plasma lipid utilization was the cause of the prolonged lipemia. Unpublished results of the investigators were quoted to the effect that intravenous injections of hyperlipemic plasma into young and old individuals resulted in similar disappearances of the chylomicrons. Hence differences in fat absorption in the two age groups seem to be responsible for the very different fat tolerance curves recorded.

According to present concepts of fat digestion and absorption, some fat is hydrolyzed and absorbed as fatty acids and transported through the portal circulation. The other part of the fat is apparently absorbed as neutral fat, its dispersion effected by bile and partial hydrolysis products of the fat. Thus it may be expected that either a lack of the fat-splitting enzyme lipase or of emulsifying power in the intestine would cause a disturbance of fat absorption. Thus it was particularly pertinent that the investigators found that oral administration of lipase or of

the detergent Tween 80 to the older persons caused a reduction in the chylomicron count to nearly the levels of the young group.

The contribution of these workers is at least significant to the study of biochemical changes in old age, but even more significantly may be related to the genesis of arteriosclerosis. How significant the findings may be to the latter problem depends on the possible relation of chylomicronemia to initiation of the atherosclerotic lesion. The reports of W. C. Hueper (*Arch. Path.* **28,** *510* (*1939*); **39,** *117* (*1945*)) and J. R. Moreton (*Science* **106,** *190* (*1947*); **107,** *371* (*1948*)) are cited to support a positive relation between these factors. These workers indicate that macromolecular substances, such as chylomicrons, can damage the artery and lead to atherosclerosis.

Becker, Meyer, and Necheles state conservatively that if the chylomicrons play a role in atherosclerosis, then their findings may provide a physiologic basis for development of the degenerative disease arteriosclerosis. They rightly consider it premature to speculate whether administration of a detergent or the enzyme lipase could prevent or alter the course of atherosclerosis, and indicate that animal experiments may throw light on these important questions.

THE GASTRIC JUICE IN SPRUE

Investigations relating to Castle's extrinsic factor and vitamin B_{12} have been reviewed in this journal (*Nutrition Reviews* **7,** *146* (*1949*)) and it was pointed out that in pernicious anemia there appears to be a failure of a reaction which normally occurs between a substance in the food (extrinsic factor) and a substance in the normal gastric secretion (intrinsic factor). Recent observations from Castle's laboratory and from that of others also reviewed indicated that the hematopoietic activity of vitamin B_{12} in pernicious anemia is potentiated by the simultaneous administration of normal human gastric juice and that perhaps the "liver principle" and the "extrinsic factor" were identical. Thus, the effect of the gastric juice factor might be thought either to favor the absorption of the vitamin or closely related food substances or to protect the vitamin from destruction in the stomach or upper gastrointestinal tract.

The nature of the intrinsic factor, that is the factor present in normal human gastric juice but absent from the gastric juice in pernicious anemia, is unknown, although observations from Castle's laboratory a decade ago indicated that a protease active at neutral pH and derived from gastric juice exhibited chemical and physical properties like that of the intrinsic factor. In addition, the gastric juice of patients with pernicious anemia was found to exhibit a markedly depleted protease activity at neutral pH (F. H. L. Taylor, W. B. Castle, R. W. Heinle, and M. A. Adams, *J. Clin. Invest.* **17,** *335* (*1938*)).

A contribution to the understanding of the nature of the intrinsic factor has been made by H. J. Fox (*J. Clin. Invest.* **28,** *687* (*1949*)), who investigated the proteolytic activity of gastric juice at a neutral pH in patients with sprue in relapse and remission induced by injectable liver extract or folic acid. It was found that the proteolytic activity of the gastric contents in cases of sprue in remission was apparently as active as that in normal gastric juice but was greatly diminished in cases of sprue in relapse.

The fact that as in pernicious anemia there is a hematologic response in sprue following parenteral administration of folic acid, vitamin B_{12}, as well as liver extract, together with the observations of Fox and of the workers in Castle's laboratory, begins to clarify some of the relations between these two diseases and factors in their etiology which may be in common. In discussing his results, however, Fox was rightly conservative and indicated that all that could be said was that the "protease activity of the gastric juice is lessened in sprue in relapse and normal in remission."

NOTES

Letter to the Editor

Dear Sir:

In commenting on the article "Nutrition Surveys in Vienna" (*Nutrition Reviews* **7**, *242* (*1949*)), I, as a former United States Army Nutrition Officer, would like to suggest that under certain conditions accurate and valuable data concerning caloric intake may be obtained by combining several investigative methods. Nutrition surveys were conducted in Germany during the latter part of 1945 and early 1946 by the Army Medical Department Nutrition Survey Teams and the material to be presented was obtained in that work.

In determining the amount of food actually eaten by the average individual of each of the various food ration categories it was assumed that the official ration issued was the actual minimal amount of food available and that it was entirely consumed. This was always verified at the ration board headquarters, at the agriculture section, and finally by questioning an adequate percentage of the consumers themselves. Information about any extra food available was obtained by study of the twenty-four-hour dietary histories. In most cases these were obtained by impartial Nutrition Officers who interrogated representatives of the many ration categories in a friendly manner usually in the native language, with which most of the Officers were familiar. Sample dishes were used to estimate the quantities. Extra amounts of foods, particularly vegetables, fruits, and especially potatoes were brought to light in this manner.

For example, the following values were obtained in different selected locales and each illustrates an interesting point. (See Table on next page.)

At Kassel it was determined that the populace was eating potatoes that had been issued for future consumption and at the rate of use the supplies would be depleted within four to six weeks from time of survey. Any unimpounded potatoes from the Kassel rural areas were being smuggled into the nearby British Zone where food riots were prevalent at the time, otherwise the caloric intake would probably have been more than the 182 calories in excess of the issued ration. As a result, food supplies were arranged in the U. S. Zone to avoid future trouble in this area.

The Berlin survey was one of a series that led to a gradual increase in rations as much as was possible under the conditions existing in the city at that time.

The more rural areas such as in Bad Homburg and Heilbronn yielded a much larger caloric difference between the issued and eaten food rations. These values represented the caloric intake for the majority of the German people and approached or exceeded the 2000 calorie minimal subsistence value set for the occupation. They also tended to refute the statement attributed to Senatory Wherry that there was mass starvation in the U. S. Zone of Germany. It must also be considered that the values obtained were averages and possibly did not always apply to members of families or groups that usually interchanged food supplies. However, they were indicative for the population as a whole. Regardless of location it was found that the aged suffered most, possibly because of their physical inability to obtain food in excess of officially issued rations.

The Zeilsheim Displaced Persons Camp offered another type of study. Complaints of undernourishment were investigated but despite lack of cooperation on the part of the camp members it was found that the official ration was in all probability being served and that extra rations were being drawn. In fact many of the individuals were overweight. Later the camp proved to be the hub of a flagrant black-market center.

The Ziegenhain Civilian Detainee Camp #95 was found to have an official ration of 1750 calories but this had not been

PLACE	DATE	NORMAL CONSUMER RATION IN CALORIES		
		Official issued ration	Ration actually eaten	Difference
Kassel	Feb. 1946	1607	1789	+182
Berlin	Dec. 1945	1193	1379	+186
Bad Homburg	Jan. 1946	1559	1897	+338
Heilbronn	Feb. 1946	1607	2019	+412
Zeilsheim Displaced Persons Camp	Nov. 1945	2344	2252*	+
Ziegenhain Civilian Detainee Camp #95	Jan. 1946	1750	1487	−263
			Hospital 2651	+901
U. S. Zone Average	Jan. 1946	1600	1920	+320

* Supplemented with numerous Red Cross boxes and other foods.

actually reached. For many months the caloric intake had been much lower by several hundred calories and as a result about one half of the camp was usually on the hospital ration. In this manner, fatal deterioration was prevented but undernourishment was common. These men had practically no additional food sources. Adequate arrangements were then made to correct these conditions in the camp.

In all of these studies the physical examinations and weight and height data correlated well with the caloric intake of the individuals as determined by a combination of methods in a populace more or less restricted as to source of food supplies.

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Diet and Lead Poisoning

The relation between diet and the liver lesions sometimes found in animals poisoned with lead has been studied by H. Chiodi and A. F. Cardeza (*Arch. Path.* **48,** *395 (1949)*). The authors fed rats by stomach tube, the composition of the diet being altered in a number of ways. The chief alteration was increasing the fat content to 40 per cent. Some of the animals were given, also by stomach tube, 2 ml. of a 4.5 per cent solution of lead acetate. The remainder of the animals, given no lead, served as controls. In addition to changes in fat content, casein, methionine, and other lipotropic agents were quantitatively altered in the diet. Unfortunately, there were so many combinations of nutrients fed that each group of rats was small in number so that conclusions are difficult to justify. Nevertheless, the rats given the 40 per cent fat diet with lead all died within one to three weeks and all showed pathologic liver changes. When the diet was high in carbohydrate rather than in fat, little if any hepatic damage was observed.

The characteristic pathologic changes were those of increase in size of cells and nuclei, fatty infiltration often associated with degeneration, and necrosis of cells. It should be noted that in addition to a high-fat content the protein content was only 14.7 per cent and that there was no source of "B vitamins" or of ascorbic acid in the basal diet producing the hepatic lesion. It was further observed by the authors that casein and brewers' yeast added to the diet or casein with lipotropic agents (choline, inositol, tocopherol, and methionine) prolonged life and diminished the hepatic changes observed.

It would seem possible to interpret these studies as indicating that the feeding of rats with a purified diet which was high in fat and somewhat low in protein and the "B vitamins" led to increased susceptibility to lead poisoning.